IN SITU EXAMINATION OF WHITE CLOVER AND PERENNIAL RYEGRASS ROOTS INOCULATED WITH FUNGAL PATHOGENS

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ABSTRACT

A minirhizotron-borescope system was used to directly measure the effect of two fungal pathogens, *Cylindrocladium scoparium* Morgan and *Fusarium crookwellense* Burgess Nelson & Tousson, on the root growth of white clover (*Trifolium repens* L.) and perennial ryegrass (*Lolium perenne* L.). This technique allowed roots to be counted *in situ* over a continuous time period without disturbing plant growth and function. Both fungal pathogens decreased clover and ryegrass root count numbers and reduced total plant dry weight. The pattern of clover root growth differed for each pathogen as *C. scoparium* decreased the total clover root count but *F. crookwellense* only reduced the rate of increase of the total clover root count.

Keywords: *Trifolium repens, Lolium perenne,* roots, soilborne fungal pathogens, minirhizotron-borescope

INTRODUCTION

The study of soilborne root pathogens has been hampered by the difficulty in making *in situ* observations on root systems obscured by the soil matrix (Rush *et al.* 1984). Development of the minirhizotron-borescope system facilitates observation of root growth and distribution (Bohm 1979). This method uses minirhizotron tubes placed in soil with a window to the root system provided by a borescope camera that is inserted into the tubes so that roots intersecting the tubes can be directly observed (Upchurch and Ritchie 1983; Taylor 1987). The clear acrylic observation tubes which allow the extendable camera to be inserted into the soil are illuminated by fiber optic light guides. The method provides a technique for root observation in a natural environment that can be used repeatedly over a continuous time period (Upchurch and Ritchie 1983).

The minirhizotron-borescope was developed for use in agronomy to quantify the dynamics of root growth and water uptake of important crops. However, Rush *et al.* (1984) applied this system for *in situ* observation of *Phymatotrichum omnivorum* (Shear) Duggar, an important phytophagous root pathogen of cotton and soybean crops. Fungal strands and root necrosis were observed on roots two weeks before above ground symptoms were visible on the plant. Similarly, the system has provided information on the interactive effect of salinity and *Phytophthora parasitica* Dastur root rot, on root growth and senescence in tomato (*Lycopersicon esculentum* Mill., Snapp and Shennan 1994).

Two fungi isolated from pasture roots, *Cylindrocladium scoparium* Morgan and *Fusarium crookwellense* Burgess Nelson & Tousson, were found to be pathogenic to both white clover and ryegrass in laboratory and pot trials (Waipara *et al.* 1996a; 1996b). These root-colonising fungi are common in North Island pastures (Waipara 1997) and were therefore selected for inoculation onto clover and ryegrass roots.

METHODS

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A pilot trial was undertaken to ascertain if the minirhizotron-borescope system could be used to measure the effect of pasture root pathogens *C. scoparium* and *F. crookwellense* on the growth of white clover and perennial ryegrass roots. Twelve plastic containers (30 cm deep x 25 cm wide) were placed on watering trays, and a clear plexiacrylic minirhizotron tube (50 cm in length, 2.5 cm diameter) was inserted in each container at a 45º angle, so that half the minirhizotron tube was inside the container to a depth of 25 cm. The minirhizotrons were marked with three transect lines with permanent coloured markers. The transect lines were drawn vertically for 25 cm down the length of each tube, with a different colour so that a line was drawn on the top, right and left side of each tube. Horizontal lines (2.5 cm apart) were then drawn across the minirhizotrons to link each transect line creating 10 sections on each tube. Each container was then filled with Horotui sandy loam soil previously passed through a 2 mm sieve and the soil then compacted around the minirhizotrons.

Twenty day old plants of perennial ryegrass (cv. Nui; endophyte-free) and white clover (cv. Huia) germinated from seed were transplanted into the soil so that six of the containers were each planted with three clover plants and six each with three ryegrass plants. These plants were left to grow for a further 20 days before being inoculated with conidial suspensions of *C. scoparium* or *F. crookwellense*, using a method outlined by Waipara *et al.*(1996a). Plants in each container were inoculated with 500 ml of conidial suspensions (10^8 conidia/ml), produced by growing each fungus on potato carrot agar at 25ºC for 3 weeks. The suspension was pipetted into the soil of each container up to a depth of 10 cm (measured with a marked pipette) and all containers were inoculated three times, 3 days apart, to ensure dissemination of conidia through the soil. Four containers (two clover and two ryegrass) were inoculated with sterilised conidial suspensions to act as controls. Plants were maintained in a glasshouse at 16-20ºC for the duration of the trial.

Twenty days after transplanting, the first root count measurements were undertaken. A root viewing borescope was used to count, at 7 day intervals, the number of roots observable at the minirhizotron tube-soil interface. The borescope was moved down all three vertical transect lines and the total number of intersecting roots were counted for each 2.5 cm section length of each tube. Root counts were made in eight out of the ten sections, the first and last 2.5 cm sections being excluded from the counts to avoid edge effects. Root measurement was continued for 12 weeks after the initial reading. Five weeks after transplanting, the soil was inoculated with fungal suspensions. Roots were then measured twice weekly for four weeks after inoculation to observe any rapid changes in root growth, after which counts were again undertaken at weekly intervals. At each observation date all roots counted were also mapped by drawing each visible root onto a transect data map to monitor root extension (root length mm) and root senescence in the same eight sections along each transect line. Different coloured pens were used to trace new root growth (all roots previously not drawn) as well as root death (disappearance or browning of roots drawn earlier). The length associated with each colour on a root map was calculated to obtain the accumulated totals of root growth and death over time, and the net root growth.

At the completion of the trial, surface-sterilised 3 mm root segments (20 from each plant) were plated onto water agar to reisolate the inoculated fungi and the remaining root and shoot components were dried in an oven for 48 hr at 60ºC to determine dry weight yields. All data were analysed by analysis of variance using LSD tests for separation of means.

RESULTS

Both *C. scoparium* and *F. crookwellense* had a visible impact on the total number of roots counted along each minirhizotron transect and the total dry weights of both clover and ryegrass. At the completion of the trial the total root count of both clover and ryegrass plants inoculated with the two fungi was less ($P < 0.01$) than that of the control plants (Figs. 1 and 2). Initially there was no difference in root numbers among the three treatments and all mean root count numbers increased in the first three measurements. However, after fungal inoculation of the soil at day 30, the clover root counts of inoculated plants initially decreased compared to the control treatments (Fig. 1). Mean

ryegrass root counts still increased after inoculation but the increase was lower for inoculated plants (Fig. 2). Overall, the total root counts of all plants increased over the 95 days but this increase was greater in control treatments. There was no difference ($P>0.05$) between the root counts of plants inoculated with *C. scoparium* and *F. crookwellense* which indicates they were equally pathogenic to these plants under the experimental conditions.

Clover root growth, as measured by the net total root length, was less in soil inoculated with *C. scoparium* or *F. crookwellense* (Fig. 3). After 95 days root growth in

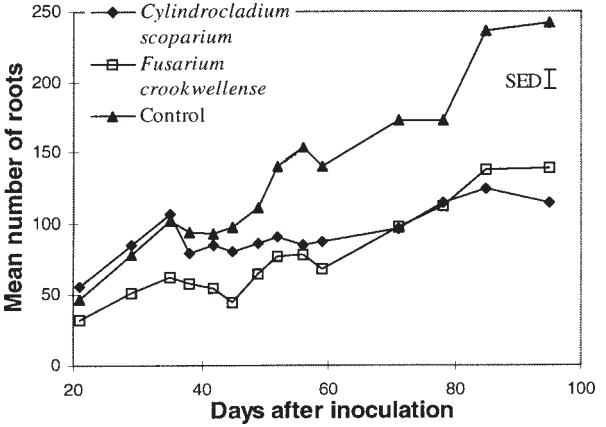


FIGURE 1: Mean root numbers counted with the minirhizotron-borescope observation system for white clover plants inoculated with *C. scoparium* and *F. crookwellense.*

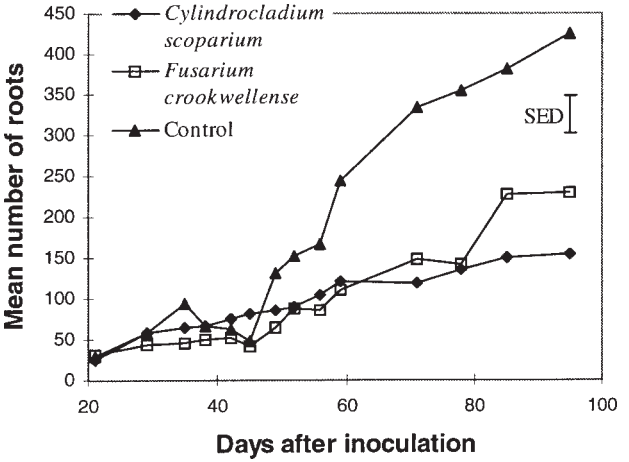
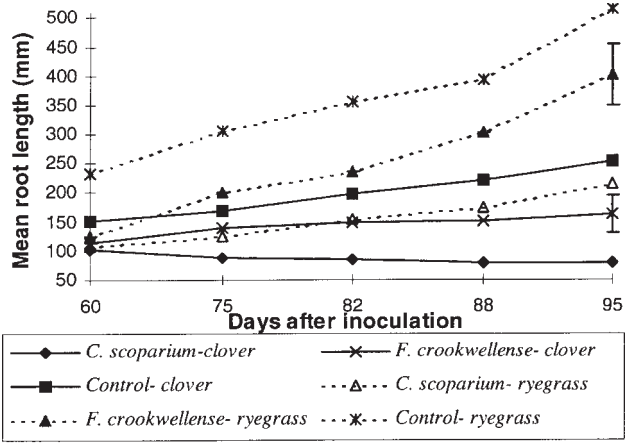


FIGURE 2: Mean root numbers counted with the minirhizotron-borescope observation system for perennial ryegrass plants inoculated with *C. scoparium* and *F. crookwellense.*

control soils had exceeded 200 mm, which was more growth ($P < 0.05$) than for roots inoculated with *F. crookwellense* (164 mm) or roots inoculated with *C. scoparium* (80 mm). Root growth of both control plants and plants inoculated with *F. crookwellense* increased over 95 days, while root growth of plants inoculated with *C. scoparium* decreased over this period.

Mean root growth of ryegrass in uninoculated soil reached 516 mm at day 95 which was greater ($P < 0.01$) than in soil inoculated with either *C. scoparium* (216 mm) and *F. crookwellense* (402 mm) (Fig. 3). Ryegrass root growth was higher for all treatments compared to clover growth. The mean root growth of all three ryegrass treatments increased over the 95 day period, although this increase was much less in the inoculated soils.

The two pathogens also reduced both clover and ryegrass dry weights. After 95 days white clover plants grown in soil inoculated with *C. scoparium* had lower mean dry shoot



Error bars represent ryegrass SED = 107, and clover SED = 64.1

FIGURE 3: Mean clover and ryegrass root length (mm) of uninoculated control plants and plants inoculated with fungi.

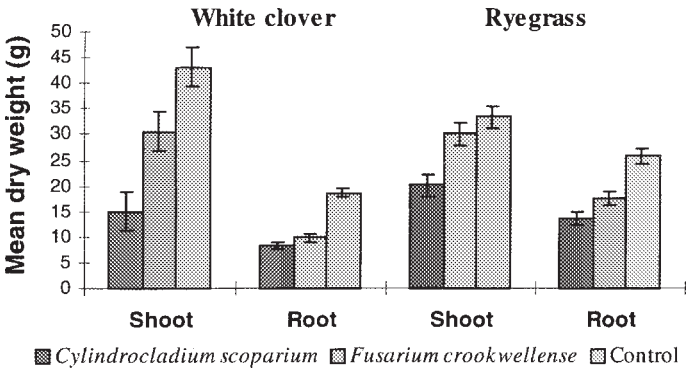


FIGURE 4: Mean dry weights of white clover and ryegrass plants inoculated with *C. scoparium* and *F. crookwellense* and uninoculated plants.

and root weights compared to control plants (Fig. 4). Results were similar for *F. crookwellense* except that no statistically significant effect on shoot weight was detected. (Fig. 4). Similar results were obtained for ryegrass plants, as the mean dry root weight was lower in soil inoculated with *C. scoparium* and *F. crookwellense* than in uninoculated soil.

Cylindrocladium scoparium and *F. crookwellense* were reisolated from root segments of both plant species which confirmed them as the causal agents of root disease. Both species were absent from control segments.

DISCUSSION

Both pathogens reduced clover and ryegrass root numbers and total plant dry matter. The reduced root numbers observed for inoculated plants corresponded with the reduced yields obtained at the end of this experiment. Ryegrass root growth was affected by these two pathogens in that root growth was reduced and although root growth continued to increase, it was at a slower rate than the controls. This contrasted with clover roots inoculated with *C. scoparium* where total root growth decreased over the measurement period.

Burgess *et al.* (1994) reported that *F. crookwellense* was mildly pathogenic to wheat roots, but this is the first report of this fungus affecting the root turnover and yields of pasture plants. Although *C. scoparium* has been previously reported to be pathogenic to pasture plants (Waipara *et al.* 1996a), the deleterious effect on root turnover had not been assessed. These results indicate that fungal root pathogens can be harmful to pasture productivity. Fungal pathogens could contribute to the reported short-term clover survival in perennial pastures. Both *C. scoparium* and *F. crookwellense* are frequently isolated from many North Island pastures where clover decline has occurred.

The minirhizotron-borescope system was successfully used to directly measure the effect of fungal pathogens on the root growth of pasture plants. This system was a useful alternative to destructive sampling methods as it enabled root numbers to be measured *in situ* over a continuous period without disturbing plant growth and function. The examination of pasture root pathogens in field conditions has to date been difficult and therefore largely ignored. Future research using this system could be applied in pasture, allowing root growth and pathogens to be monitored. More extensive trials will be undertaken in the future to determine the seasonal root turnover of pasture plants in the presence of soilborne pathogens under field conditions.

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